

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Currently amended) A method for producing embryoid bodies (EBs) from pluripotent cells comprising

(i) obtaining a liquid single cell suspension culture of pluripotent cells;
(ii) collecting and suspending the cells in a container to a density of about 0.5×10^6 to 5×10^6 cells/ml;

(iii) rocking the container containing the liquid single cell suspension culture thereby generating cell aggregates; and

(iv) diluting the suspension, and further rocking a container containing the suspension until formation of EBs; wherein the final concentration of EBs in the suspension culture is about 500 EBs/ml;

wherein the pluripotent cells are selected from the group consisting of embryonic stem (ES) cells, primordial germ (EG) cells and pluripotent adult stem cells.

2. (Currently Amended) The method of claim 1, wherein prior to step (i) the cells are cultured on embryonic mouse fibroblasts (feeder cells).

3. (Previously Presented) The method of claim 1 or 2, wherein said pluripotent cells are embryonic stem (ES) cells.

4. (Previously Presented) The method of claim 3, wherein said cells are obtained from a murine ES cell line.

5. (Currently Amended) The method of claim 1, wherein the culture medium in any or all of the steps is Iscove's Modified Dulbecco's Media (IMDM), 20% fetal calf serum (FCS) and ~~5% CO₂~~.

6. (Currently Amended) The method of claim 1, wherein the culture conditions in any or all of steps (i) through (iii) comprise 37°C, 5% CO₂ and 95% humidity.

7. (Previously Presented) The method of claim 1, wherein said culture of pluripotent cells has a concentration of about 1×10^6 to 5×10^6 cells/ml.

8. (Previously Presented) The method of claim 1, wherein the suspension in step (iii) is cultured for about 6 hours.

9. (Currently Amended) The method of claim 1 [[8]], wherein the suspension is cultured for about 16 to 20 hours.

10. (Previously Presented) The method of any one of claims 1, 8 or 9, wherein the suspension in step (iv) is cultured in T25 flasks.

11. (Previously Presented) The method of claim 1, wherein said dilution in step (iv) is 1:10.

12. (Canceled)

13. (Currently Amended) The method of claim 1, further comprising diluting ~~dividing~~ the cell aggregates to the desired final concentration.

14. - 16. (Canceled)

17. (Previously Presented) The method of claim 1, further comprising culturing the EBs under conditions allowing differentiation of the EBs into cardiomyocytes.

18. (Canceled)

19. (Previously Presented) The method of claim 17, further comprising selection of cardiomyocytes by use of one or more selectable markers or agents or both.

20. (Previously Presented) The method of claim 19, wherein said cell is genetically engineered.

21. (Previously Presented) The method of claims 19 or 20, wherein said cell comprises a selectable marker or a reporter gene or both.

22. (Previously Presented) The method of claim 21, wherein said cell comprises a selectable marker gene operably linked to a cell type-specific regulatory sequence.

23. (Original) The method of claim 22, wherein said selectable marker confers resistance to puromycin.

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24. (Previously Presented) The method of claim 21, wherein said cell comprises a reporter gene operably linked to a cell type-specific regulatory sequence.

25. (Previously Presented) The method of claim 24, wherein said cell type-specific regulatory sequence of the reporter gene is the same as said cell type-specific regulatory sequence of the marker gene.

26. (Previously Presented) The method of claim 25, wherein said reporter is enhanced green fluorescent protein (EGFP).

27. (Previously Presented) The method of claim 21, wherein said marker gene and said reporter gene are contained on the same recombinant nucleic acid molecule.

28. (Original) The method of claim 27, wherein said marker gene and said reporter gene are contained on the same cistron.

29. (Previously Presented) The method of claim 22, wherein said cell type-specific regulatory sequence is atrial- or ventricular-specific.

30. (Previously Presented) The method of claim 29, wherein said regulatory sequence is a cardiac-specific regulatory sequence selected from the group consisting of the promoters of alpha-myosin heavy chain (alpha-MHC) and ventricular myosin light chain 2 (MLC2v).

31 - 44. (Canceled)

45. (Currently Amended) A method for producing embryoid bodies (EBs) from pluripotent cells comprising

- (i) obtaining a liquid single cell suspension culture of pluripotent cells;
 - (ii) collecting and suspending the cells in a container to a density of about 0.1×10^6 to 1×10^6 cells/ml;
 - (iii) rocking the container containing the liquid single cell suspension culture thereby generating cell aggregates; and
 - (iv) rocking the container containing the suspension until formation of EBs;
- and
- ~~(v) diluting the resultant EBs to a concentration of about 100-2000 EBs/10 ml~~

wherein the pluripotent cells are selected from the group consisting of embryonic stem (ES) cells, primordial germ (EG) cells and pluripotent adult stem cells, and wherein a 10 ml aliquot of a suspension in (ii) comprising 0.2×10^6 pluripotent cells yields sufficient EBs to seed six 20 ml suspensions each comprising 1000 EBs.

46. (Previously Presented) The method of claim 45, wherein prior to step (iii) the cells are cultured on embryonic mouse fibroblasts (feeder cells).

47. (Previously Presented) The method of claim 45 or 46, wherein said pluripotent cells are embryonic stem (ES) cells.

48. (Previously Presented) The method of claim 47, wherein said cells are obtained from a murine ES cell line.

49. (Currently Amended) The method of claim 45, wherein the culture medium in any or all of the steps is Iscove's Modified Dulbecco's Media (IMDM), 20 % fetal calf serum (FCS) ~~and 5 % CO₂~~.

50. (Currently Amended) The method of claim 45, wherein the culture conditions in any or all of steps (i) through (iv) comprise 37°C, 5 % CO₂ and 95 % humidity.

51. (Previously Presented) The method of claim 45, wherein said culture of pluripotent cells has a concentration of about 0.1×10^6 to 0.5×10^6 cells/ml.

52. (Previously Presented) The method of claim 45, wherein the suspension is cultured for about 48 hours.

53. (Canceled)

54. (Previously Presented) The method of claim 45, further comprising culturing the EBs under conditions allowing differentiation of the EBs into cardiomyocytes.

55. (Previously Presented) The method of claim 54, further comprising selection of cardiomyocytes by use of one or more selectable markers or agents or both.

56. (Previously Presented) The method of claim 55, wherein said cell is genetically engineered.

57. (Previously Presented) The method of claims 55 or 56, wherein said cell comprises a selectable marker or a reporter gene or both.

58. (Previously Presented) The method of claim 57, wherein said cell comprises a selectable marker gene operably linked to a cell type-specific regulatory sequence.

59. (Previously Presented) The method of claim 58, wherein said selectable marker confers resistance to puromycin.

60. (Previously Presented) The method of claim 57, wherein said cell comprises a reporter gene operably linked to a cell type-specific regulatory sequence.

61. (Previously Presented) The method of claim 60, wherein said cell type-specific regulatory sequence of the reporter gene is the same as said cell type-specific regulatory sequence of the marker gene.

62. (Previously Presented) The method of claim 61, wherein said reporter is enhanced green fluorescent protein (EGFP).

63. (Previously Presented) The method of claim 57, wherein said marker gene and said reporter gene are contained on the same recombinant nucleic acid molecule.

64. (Previously Presented) The method of claim 63, wherein said marker gene and said reporter gene are contained on the same cistron.

65. (Previously Presented) The method of claim 58, wherein said cell type-specific regulatory sequence is atrial- or ventricular-specific.

66. (Previously Presented) The method of claim 65, wherein said regulatory sequence is a cardiac-specific regulatory sequence selected from the group consisting of the promoters of alpha-myosin heavy chain (alpha-MHC) and ventricular myosin light chain 2 (MLC2v).

67- 68. (Canceled)

69. (Previously Presented) The method of claim 24, wherein said cell type-specific regulatory sequence is atrial- or ventricular-specific.

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70. (Previously Presented) The method of claim 60, wherein said cell type-specific regulatory sequence is atrial- or ventricular-specific.